Laboratory experiments testing the effectiveness of the commercially available product PoCo in mitigating cyanobacterial blooms and decomposing organic matter

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Report M349
February 2009

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Content

Summary

The Dutch company WiseUse International BV has proposed using a so-called bio stimulator “PoCo” (Pollution Control) in ecological restoration of Lake Ypacarai (Paraguay). Because of lacking scientific and insufficient supporting studies, the Ministry of Economical Affairs (represented by the EVD and supported by VROM) has ascribed sponsoring to WiseUse under the condition that the Aquatic Ecology and Water Quality Management group of Wageningen UR would supervise the scientific solidity of the proposed tests.

In this study results of laboratory assays are reported testing the performance of PoCo regarding the alleged enhanced mineralization of organic matter in the water phase and sediment, the inhibition of cyanobacterial growth (as a clear measure counter acting the symptoms of eutrophication) and effects on survival of the waterflea *Daphnia.*

Although it has been suggested that PoCo would be highly efficient in degrading sludge and mitigating the nuisance symptoms of eutrophication in Lake Ypacarai, this study yielded no support for these claims. Organic mater in water and sediment from Lake Ypacarai was not reduced by PoCo, nor could growth of cyanobacteria be hampered. No effect at recommended doses (0.0005 – 0.002 ml l^{-1}) was observed; only at very high dosage (1ml l^{-1}) some growth inhibition in cyanobacteria was detected. However, these concentrations appeared acutely toxic to the water flea *Daphnia magna*.

Inasmuch as no indication was obtained in favor of any of the claims regarding effectiveness or alleged working mechanism of PoCo, usage of PoCo at large scale is very doubtful.

Disclaimer
Despite strong criticism on the initial proposal by VROM, and after similar critiques from AEW, WiseUse BV, nonetheless, continued implementing a pond experiment in Lake Ypacarai. We consider the scientific set-up insufficient, have received doubtful and wishful interpretations of persons involved by email and dissociate our selves from any claims made on effectiveness of PoCo in that context. Furthermore, we were not involved in the setup and execution of any laboratory experiments in Paraguay.

Introduction

Lake Ypacarai is a 60 km^2 shallow lake (mean depth 2 m) located 30 km east of Asuncion (Paraguay). Lake Ypacarai suffers from ongoing pollution through discharge of untreated urban waste water, industrial effluents and run-off from surrounding crop fields, livestock farms, and deforested lands. The urban, agricultural and industrial effluents can contain high concentrations of nutrients, large amounts of sediment, oxygen-consuming wastes, pathogens and a whole palate of toxic substances (heavy metals, oil, pesticides). The high nutrient loads promote harmful cyanobacterial blooms and the accumulated organic matter might cause severe oxygen depletion. The variety of water quality problems can be harmful to humans and cattle, limit recreational activities, and decrease fisheries.

The progressive eutrophication, illustrated for example by 1984 average total-Nitrogen and total-Phosphorus concentrations in the lake of 0.70 (± 0.10) mg l^{-1} and 0.25 (± 0.05) mg l^{-1}, respectively (ILEC, 2008), motivated the Paraguayan authorities to undertake actions mitigating the nuisance (ILEC, 2008).

WiseUse International BV (The Netherlands) has proposed a restoration of the Lake Ypacarai through application of the biocatalyst “PoCo”, which is an abbreviation for Pollution Control. According to the manufacturers, PoCo is a biocatalyst that “…*stimulates and accelerates the growth of micro-organisms in a natural way by micro-nutriment and trace elements*” (WiseUse, 2008). The proposed underlying mechanism is that these micro-organisms either consume or outcompete the cyanobacteria and stimulate or perform the degradation of organic matter (sludge) concomitantly reducing foul odors (Mundhe and Pool, 2007; WiseUse, 2008).

However, the scientific support for these claims is very weak and pilot-tests (f.e. Mundhe and Pool, 2007) did not meet the required standards. Inasmuch as this flaw was recognized by the Ministry of VROM, the Aquatic Ecology and Water Quality Management group (Wageningen UR) was contacted for scientific support. Here we report on laboratory assays testing PoCos performance regarding:

1) Enhanced mineralization of organic matter in the water phase
2) Enhanced mineralization of organic matter in the sediment
3) Inhibition of cyanobacterial growth

and its effect on:

4) Survival of the waterflea *Daphnia.*

PoCo and mineralization of organic matter

PoCo is alleged to stimulate organic matter decomposition. We tested PoCos performance regarding enhanced mineralization of organic matter in the water and sediment from Lake Ypacaria. On March 21st 2008 we received 4.3 liters of water, one liter of phytoplankton material and three jars with lake sediment; ten days later a liter of PoCo arrived (*Figure 1*).

Figure 1: *Water and sediment form Lake Ypacarai (left panel) and PoCo (right panel).*

PoCo appeared being a dark brown (*Figure 2*), viscose liquid, which had been made from plant extracts with unspecified/unknown active ingredients. The recommended dosage of PoCo is 0.0005 – 0.002 ml l^{-1}.

Effect of PoCo on mineralization of organic matter in the water phase

The effect of PoCo on degradation of organic material in the water from Lake Ypacari was tested in 100 ml Erlenmeyer flasks that contained different concentrations of PoCo in 100 ml water from Lake Ypacarai. PoCo was tested in triplicate in the following concentrations: 0, 2×10^{-6}, 2×10^{-5}, 2×10^{-4}, 2×10^{-3}, 0.02, 0.2, 2.0, and 20 ml PoCo l^{-1}. Erlenmeyer's were closed with a cellulose-plug and incubated for 21 days in a Gallenkamp ORBI-SAFE Netwise Orbital Incubator (*Figure 2*) at a constant temperature of 24 °C, 50 rpm and a 18:6 light/dark cycle (with a maximum of 10.200 lux). One additional set of samples was frozen immediately (t=0) for later total inorganic and organic carbon (TIC and TOC) analysis, including a sample of PoCo. After incubation TIC and TOC of all samples was determined in duplicate using a Total Organic Carbon analyzer (Model 700, O.I.C. International BV).

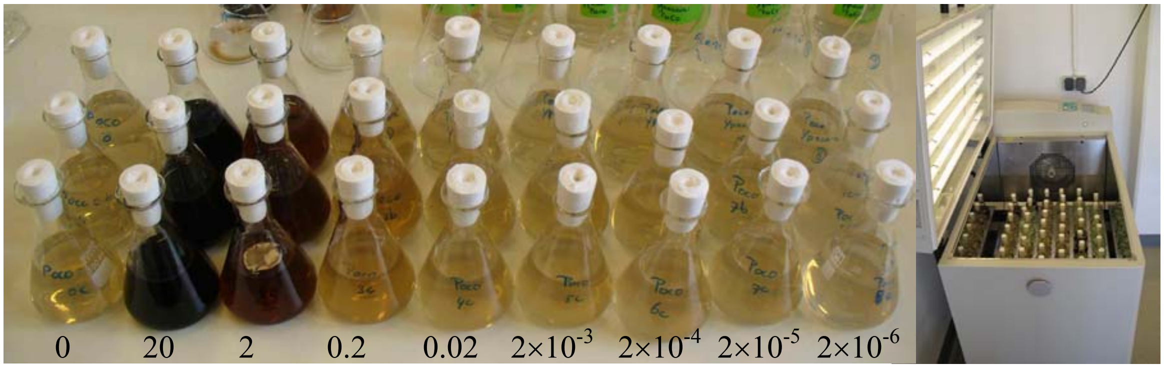

Figure 2: *Erlenmeyer flasks with Lake Ypacarai water and different concentrations (ml l^{-1}) of PoCo added (left panel) and the used incubator (right panel).*

The TOC and TIC concentrations of undiluted PoCo were 82.8 g l^{-1} and 2.7 g l^{-1}, respectively. The TOC and TIC concentrations of Lake Ypacaria water were 7.4 mg l^{-1} and 4.5 mg l^{-1}, respectively. In general after 21 days incubation in low to moderate concentrations of PoCo (i.e. 0 – 0.002 ml l^{-1}) TOC concentrations increased compared to initial values (*Figure 3*). At a high dosage of PoCo (0.02 – 20 ml l^{-1}) a decrease in TOC compared to initial concentrations could be observed. However, these values were still exceeding the TOC concentrations of the Lake water (*Figure 3*). In all incubations TIC concentrations after 21 d were higher than the initial ones (*Figure 3*). Inasmuch as there was no homogeneity in variances, TIC and TOC concentrations after the incubation period could not be compared statistically running a one-way ANOVA. Instead means were compared separately running *t*-tests in the tool pack SPSS version 16.0 (*Table 1*). Table 1 clearly reveals that TIC concentrations are significantly elevated in incubations with 0.02 ml PoCo l^{-1} added and higher, and that these treatments also differed from each other. TOC was significantly higher in the 2 and 20 ml l^{-1} treatments (*Table 1 and Figure 3*).

Table 1*: P-values of t-test comparing the mean TOC concentrations (green fields) and TIC concentrations (white fields) in Lake Ypacarai water after 21 d with different concentrations of PoCo added (ml l^{-1}). Bold numbers indicate significant differences.*

PoCo	**0**	**2×10^{-6}**	**2×10^{-5}**	**2×10^{-4}**	**2×10^{-3}**	**0.02**	**0.2**	**2**	**20**
0		0.577	0.111	0.713	0.662	0.728	**0.002**	**0.015**	**0.016**
2×10^{-6}	0.558		0.130	0.960	0.328	0.779	**0.002**	**0.016**	**0.016**
2×10^{-5}	0.921	0.611		0.337	0.077	0.110	**0.006**	**0.016**	**0.016**
2×10^{-4}	0.276	0.927	0.483		0.507	0.860	**0.006**	**0.015**	**0.015**
2×10^{-3}	0.633	0.840	0.628	0.862		0.424	**0.002**	**0.015**	**0.016**
0.02	0.234	0.780	0.462	0.082	0.380		**0.002**	**0.016**	**0.016**
0.2	0.110	0.173	0.525	**0.022**	0.405	0.774		**0.018**	**0.016**
2	**0.017**	**0.017**	**0.016**	**0.017**	**0.002**	**0.016**	**0.017**		**<0.001**
20	**0.050**	**0.050**	**0.050**	**0.050**	**0.050**	**0.050**	**0.050**	0.057	

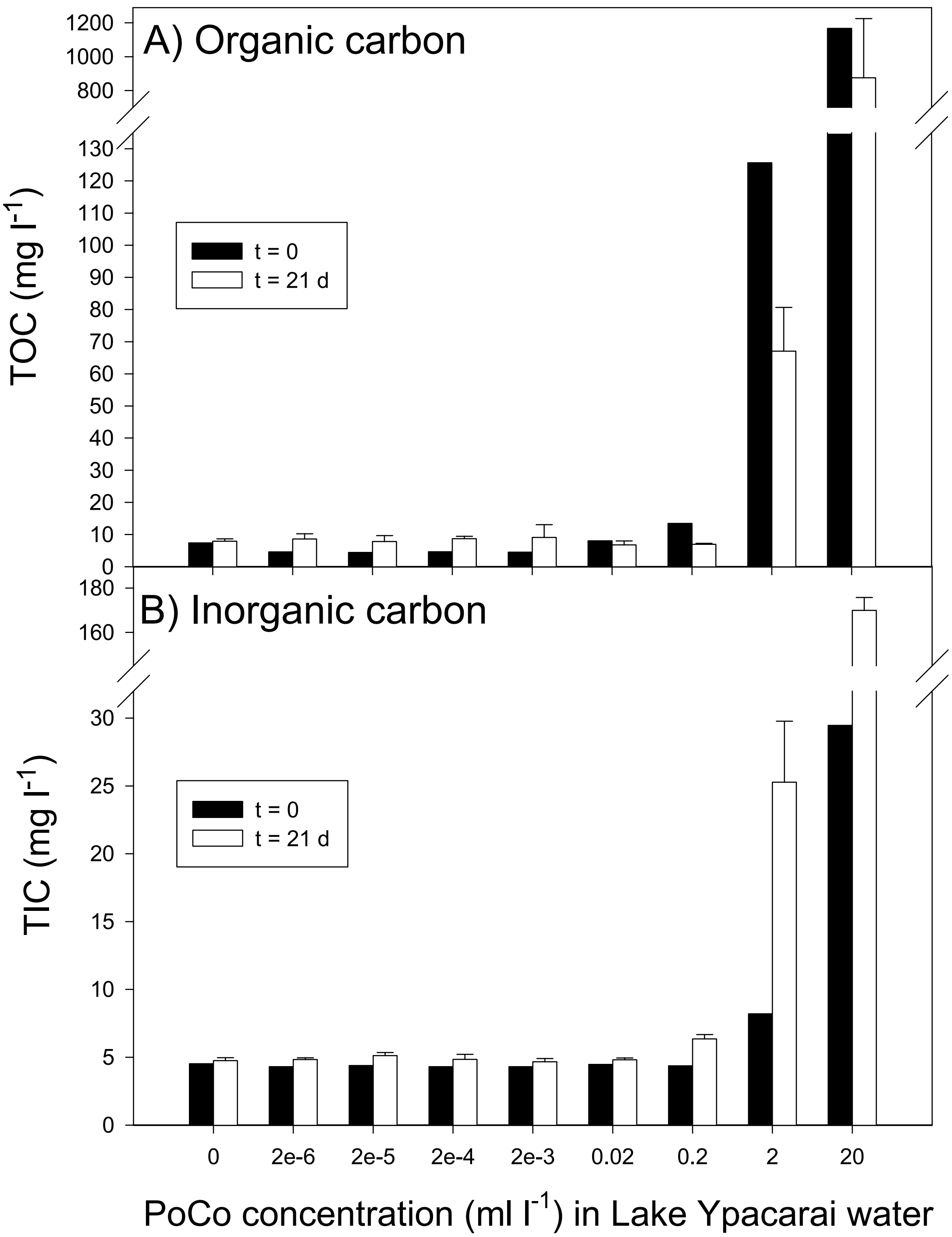

Figure 3: *Total organic carbon (TOC, mg l^{-1}; upper panel A) and total inorganic carbon (TIC, mg l^{-1}; lower panel B) in water from Lake Ypacarai with different concentrations of PoCo (0 – 20 ml l^{-1}) before (black bars) and after 21 days incubation (white bars). Error bars indicate one standard deviation (n = 3).*

At low PoCo concentrations the TOC increased during the incubation period, which could be explained from photoautotrophic growth of phytoplankton in the water as flasks appeared greenish after the incubation period. Also chemoautotrophic activity of microflora in the Lake water cannot be excluded. The TOC concentrations were, however, not significantly higher than the concentration found in the lake water without PoCo. Hence, PoCo additions in concentrations of 2×10^{-6} to 0.002 ml l^{-1} had no effect on decrease of organic matter in Lake Ypacarai water. At PoCo concentrations above 0.02 ml l^{-1} the TOC of lake water strongly increased due to the organic matter content of PoCo itself. The decrease in TOC and increase in TIC during the incubation shows that PoCo is indeed biodegradable, but after 3 weeks the TOC is still much higher than the lake water. Thus, at concentrations of 0.02 ml l^{-1} PoCo leads to an increase in organic matter instead of a decrease.

Effect of PoCo on mineralization of organic matter in the sediment

The effect of PoCo on degradation of organic material in the sediment from Lake Ypacari was tested in 100 ml plastic containers. Each container contained 20 mg (wet weight) sediment from Lake Ypacaraí, which was weighted on a analytical balance. To five plastic containers 40 ml of lake Ypacaraí water was added, to another five 40 ml of diluted PoCo (1 ml PoCo in 1liter of Ypacaraí water) was added. The 10 containers were incubated in the dark at 30^{o}C at 25 rotations per minute in a Gallenkamp ORBI-SAFE Netwise Orbital Incubator. After 21 days total dry weight was determined after drying the sediments at 105^{o}C over night. Ash weight (the inorganic component of the sediment) was determined after heating the sediment samples for 2 hours at 550 ^{o}C.

The organic fraction of the lake sediment was 7.0 (± 0.2) % (n = 5). After 21 d incubation, the organic content had slightly increased to 7.8 (± 0.1) % probably due to microflora growth. However, there was no difference in organic matter content between the sediment that had been in contact with PoCo and the sediment without this treatment (*Figure 4*, ANOVA, $F_{1,8} = 0.45$; $p = 0.522$).

There was no increase of organic matter decomposition observed when PoCo was added to the lake Ypacaraí sediment. This contradicts the alleged sludge reduction by PoCo.

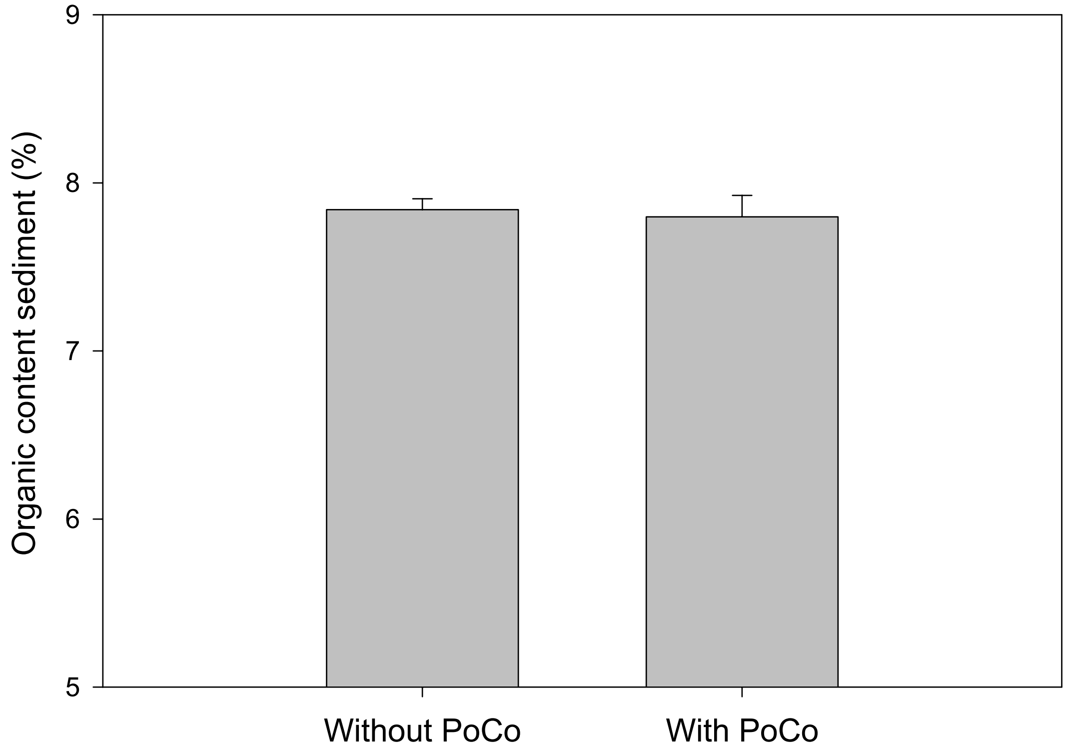

***Figure 4**: Percentage organic matter in sediments incubated with or without PoCo (1ml l^{-1}) for 3 weeks. Error bars show one standard deviation (n = 5).*

PoCo and mitigating cyanobacterial growth

The effect of PoCo on cyanobacterial growth was tested in controlled laboratory experiments with the common cyanobacterium *Microcystis aeruginosa*, which was the dominant species in the Lake Ypacarai phytoplankton sample.
First, the potential of PoCo in inhibiting the growth of *M. aeruginosa* was estimated. The recommended dose is between 0.0005 and 0.002 ml l^{-1}. However, to obtain information at what concentration inhibition might occur a far broader ranged was applied in the pilot assay. In this pilot essay PoCo was tested in the following concentrations: 0, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 0.01, 0.1, and 1 ml l^{-1}.
The experiment was conducted in 100 ml Erlenmeyer flasks that contained 50 ml of sterile algal growth medium (Lurling & Beekman, 2006). To each flask *M. aeruginosa* was inoculated at identical concentrations of 17 µg l^{-1}. The Erlenmeyer flasks were closed with cellulose plugs and incubated in a Gallenkamp ORBI-SAFE Netwise Orbital Incubator. The temperature was held constant at 24°C, flasks were gently stirred at 25 rpm and experienced a 18:6 h light:dark rhythm. The light:dark cycle was programmed in such a way that light intensity increased gradually to a maximum of 130 µmol quanta m^{-2} s^{-1} and subsequently decreased again to darkness, which resulted in a daily average light intensity of ~ 57 µmol quanta m^{-2} s^{-1}.

After 17 days samples were taken and analyzed for their chlorophyll-*a* content (µg l^{-1}) using a PhytoPAM phytoplankton analyzer (Heinz Walz GmbH, Effeltrich, Duitsland).

The pilot experiment showed that PoCo did not hamper growth of *M. aeruginosa* in concentrations up to 0.1 ml l^{-1} and that only the extremely high dose of 1 ml l^{-1} inhibited growth (*Figure* 5).

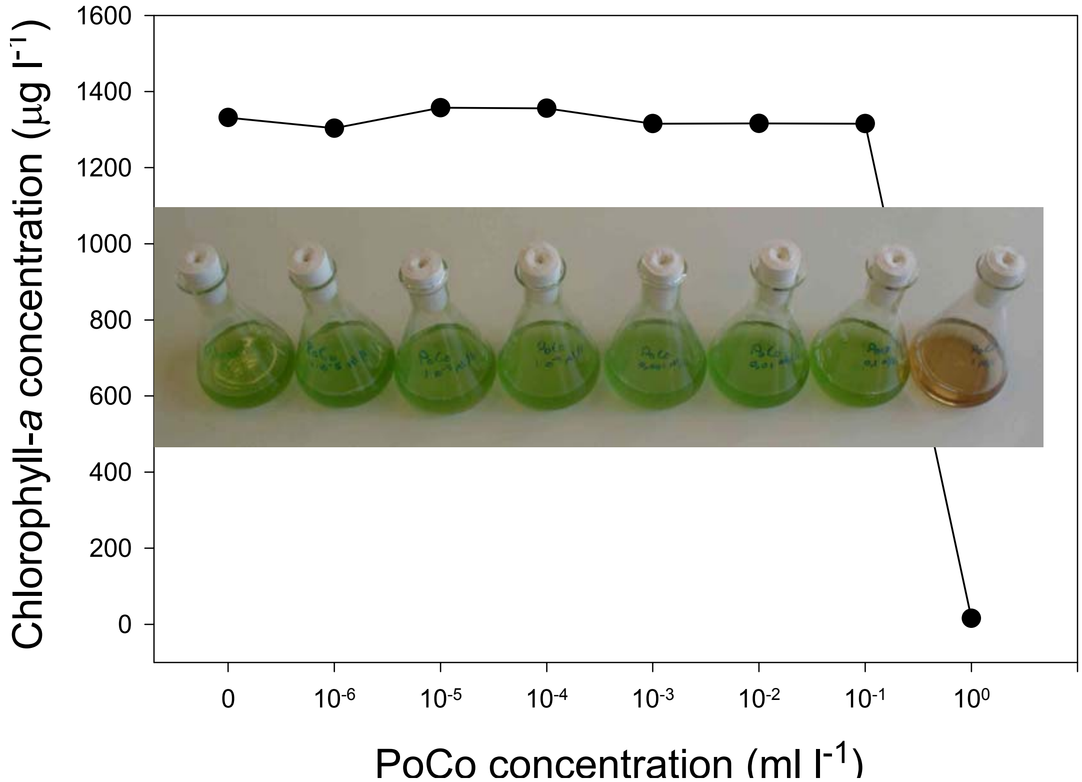

Figure 5: *Chlorophyll-a concentrations of Microcystis aeruginosa cultures after 17 days in different concentrations PoCo. Also include a photograph of the cultures after 17 days.*

Based on the results of the pilot assay a second experiment with PoCo was performed. PoCo was tested in concentrations of 0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 ml l^{-1}. The concentrations were tested in triplicate using the same methodology as described above. The initial *M. aeruginosa* concentration was 14 µg l^{-1}. Initially and after 3, 5, 7, 11, 13 and 16 days the chlorophyll-*a* concentrations were measured.

Growth of *M. aeruginosa* was inhibited slightly at PoCo concentrations of 0.6 ml l^{-1} and higher (*Figure 6*). A repeated measures ANOVA indicated a significant time effect ($F_{6,84}$ = 1967; $p < 0.001$), a significant PoCo effect ($F_{6,14}$ = 12.8; $p < 0.001$) and a significant time × PoCo interaction ($F_{36,84}$ = 7.95; $p < 0.001$). Tukey post-hoc comparison test revealed three homogeneous groups: 1) 0 – 0.6 ml l^{-1}, 2) 0, 0.6 and 0.8 ml l^{-1}, and 3) 0.6, 0.8 and 1 ml l l^{-1}. Hence, only the highest dose differed significantly from the controls. However, it should be noted that in all treatments very high blooming concentrations between 900 and 1400 µg chlorophyll-*a* l^{-1} were reached (*Figure 6*).

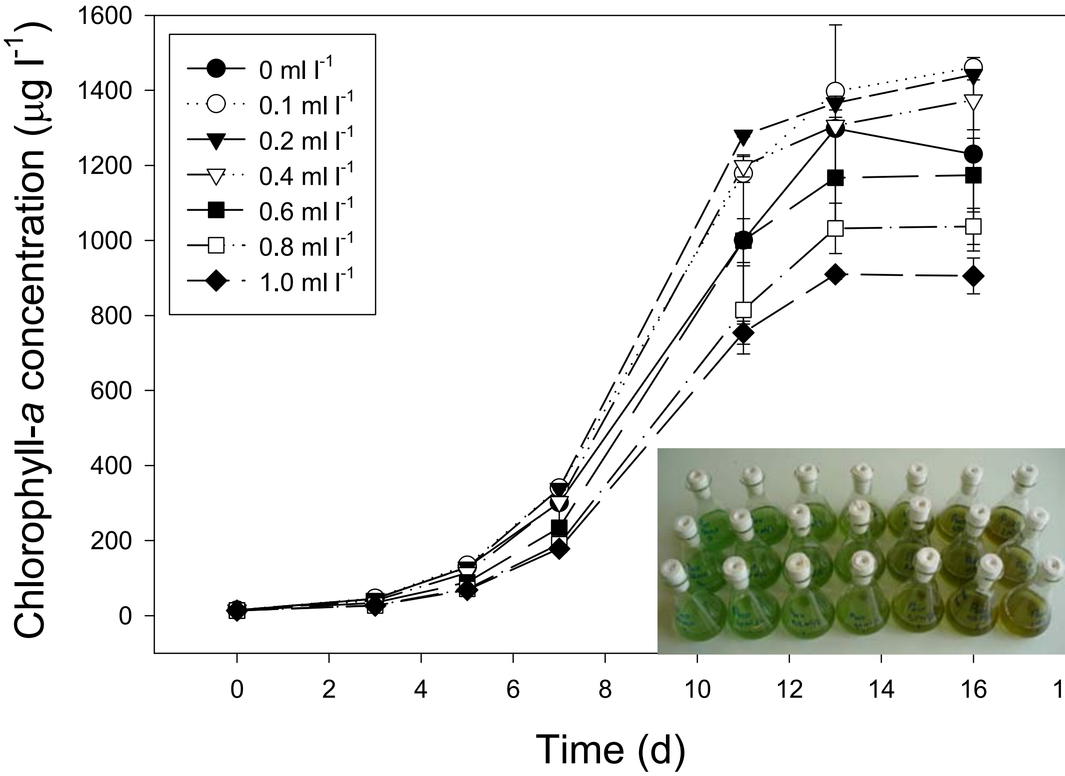

Figure 6*: Course of the chlorophyll-a concentrations of Microcystis aeruginosa cultures exposed to different concentrations of PoCo (0 – 1 ml* l^{-1}*). Error bars indicate one standard deviation (n = 3). Also included is a photograph of the cultures after 16 days incubation.*

The results of these experiments clearly demonstrated that PoCo did not inhibit growth of *M. aeruginosa*. At the recommended PoCo dose between 0.0005 and 0.002 ml l^{-1}, i.e. 5×10^{-4} and 2×10^{-3} ml l^{-1}, no effect on *M. aeruginosa* was found. Only at a very high dose of 1 ml l^{-1} some indication of growth inhibition was obtained, which could easily be caused by shading as the water was very dark. The difference between the pilot experiment and the second experiment at this highest dose is most likely caused by pipetting variations as a result of the viscosity of the undiluted PoCo. The *M. aeruginosa* exhibited excellent growth in both experiments reaching similar high chlorophyll-*a* concentrations.

Effect of PoCo on the waterflea *Daphnia*

The effect of different concentrations of PoCo on survival of the waterflea *Daphnia magna* was tested in jars that contained 800 ml RT-medium (Tollrian, 1993). To each jar 15 *D. magna* neonates (1-day old) were added without the addition of food. PoCo was tested in triplicate in the following concentrations: 0. 0.002, 0.1, 0.2, 0.4, 0.6 and 0.8 ml l^{-1}. The jars were placed in a Gallenkamp ORBI-SAFE Netwise Orbital Incubator with no light and at a constant temperature of 22 °C. After 48 hours the jars were filtered over a zooplankton filter and dead *Daphnia* were counted. Living *Daphnia* were placed back in the jars and after 96 hours the amount of dead *Daphnia* was counted again. The LC_{50} concentration (i.e. the concentration at which 50% of the animals had died) was calculated from non-linear regression (3 parameter sigmoid model) using the program SigmaPlot version 6.00. The turbidity of the water with different PoCo concentrations was measured using a HACH 2100P turbidity meter.

After 48 h only few animals had died in PoCo concentrations up to 0.4 ml l^{-1}, about 30% had died in 0.6 ml l^{-1} and 60% animals did not survive in 0.8 ml l-1. Two days later, after 96 h, mortality was increased further (*Figure 7*). The $LC_{50\text{-}48\,h}$ and $LC_{50\text{-}96\,h}$ values were 0.73 ml l^{-1} (r^2_{adj} = 0.969) and 0.65 ml l^{-1} (r^2_{adj} = 0.939), respectively.

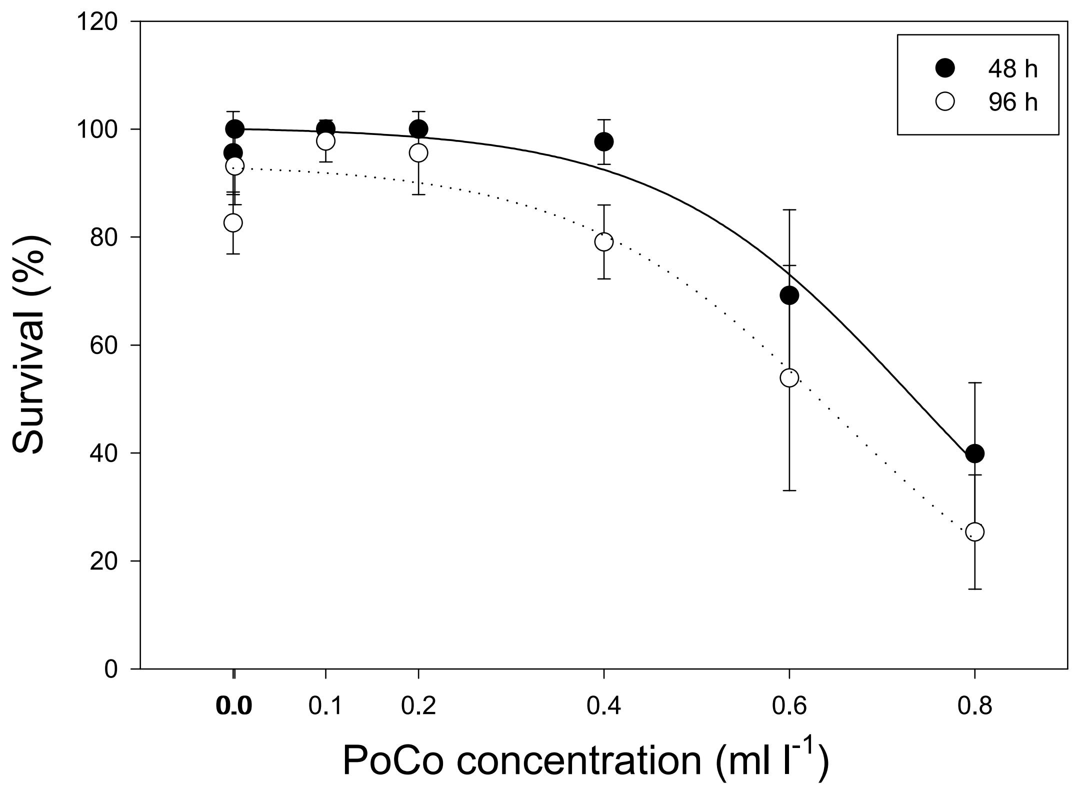

Figure 7*: Survival of the waterflea Daphnia magna after 48 h (black symbols) and 96 h (white symbols) in different concentrations of PoCo. Error bars indicate 1SD (n =3).*

The turbidity of the water increased with increasing amounts of PoCo added. Where control water (0 ml l^{-1}) had a turbidity of 0.7 NTU, turbidity of 0.1, 0.2, 0.4, 0.6 and 0.8 ml PoCo l^{-1} were: 1.5, 2.3, 3.5, 5.0 and 7.5 NTU.

The $LC_{50\text{-}96h}$ of 0.65 ml l^{-1} is close to the value where some slight growth inhibition in the cyanobacterium *M. aeruginsa* was observed. Hence, it is not advisable using doses in this order of magnitude. Moreover, the water turns dark brown at these concentrations undoubtedly causing aesthetic problems too.

Conclusion

It has been suggested that PoCo would be highly efficient in degrading sludge and mitigating the nuisance symptoms of eutrophication in Lake Ypacarai. This study yielded no support for these claims. Organic matter in water and sediment from Lake Ypacarai was not reduced by PoCo, nor could growth of cyanobacteria be hampered. No effect at recommended doses was observed; only at very high dosage some growth inhibition in cyanobacteria was detected. However, these concentrations appeared acutely toxic to the water flea *Daphnia magna*.
Controlled laboratory experiments are not designed for mimicking the natural situation, but are extremely well suited for examining underlying mechanisms and testing hypotheses. Inasmuch, as no indication was obtained in favor of any of the claims regarding effectiveness of PoCo, usage of PoCo at large scale is very doubtful.
The product is alleged being a biocatalyst that "…*stimulates and accelerates the growth of micro-organisms in a natural way by micro-nutriment and trace elements*". Even in the most positive scenario in which a rich microflora growth is stimulated, the microbes will not be able to "destroy" any heavy metals. Moreover, the microbes are on the menu of many grazers, such as heterotrophic nanoflagellates (Sheldon *et al*., 1986). This implies that even under this hypothetically very positive scenario, nutrients such as phosphate will not be fixed in the microbes or removed from the system, but will become available to cyanobacteria through trophic interactions. Off course, the cyanobacteria themselves are microbes. However, this study yielded no support that cyanobacteria were being stimulated to accelerate their growth.

For Lake Ypacarai it remains essential that alternative measures will be studied. Inflow of nutrients and pollutants should be stopped and the internal load tackled. Here, effect of combined dredging, flocculants, P-fixatives, compartmenting, sediment capping and macrophyte stockings could be considered.

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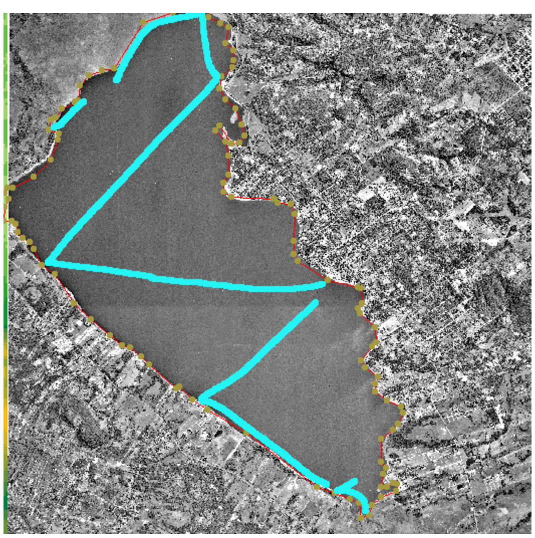
Appendix 1

From 19th to 27th October 2007, a field trip to Lake Ypacarai was undertaken with the objectives:

- Get to know people involved
- Find out their ideas concerning the lake, its problems, possible solutions, main questions and necessary research.
- Find out if there is interest a sandwich PhD
- Get a general idea of the lake + basin
- Transparency
- Thickness of sediment layer
- Consistency sediment layer
- Inlet / outlet
- Sample of algae / algal bloom

The depth of the lake could be measured using the echo sounder (left panel), unfortunately not the thickness of the sediment layer because of its composition. Some indication could be obtained from core samples (right panel).



The trajectory traveled on the Lake is depicted in the figure to the left in blue. The mean depth turned out to be 2.16 (± 0.62) m.

One the lake surface dwelling cyanobacteria (*Microcystis aeruginosa*) were encountered.